found in the hemolymph of mated wild-type females where it is degraded into smaller non-functional peptides [19]. Taken together with the almost ubiquitous distribution of the SPR (see above) to many peripheral neurons and specific parts of the VNC and the brain [13], these findings suggest that SP may have additional effects. The dsx^+ /fru^+/ppk^+ neurons are very likely the primary targets. But SP entered into the hemolymph may in addition act by modulating the activity of the SP circuitry by modifying sensory input via binding to afferent nerve axons and/or directly on presynaptic terminals [7,14–16]. Finally, stimulation of juvenile hormone synthesis and inducing the immune response in various organs very likely occurs via SP entered into the hemolymph [7].

In sum, Rezaval *et al.* [3] have identified ascending neurons that target the brain, local interneurons and descending neurons that innervate the reproductive system. All express *dsx* and are involved in the SP response, hence confirming that *fru* and *dsx* are involved in establishing sexual dimorphic features of neural circuitry in fruit flies [1]. This is a major step towards understanding the complex function of a fascinating small male peptide. It will be interesting to learn how *fru* and *dsx* shape the development of the relevant neuronal circuitries.

References

1. Siwicki, K.K., and Kravitz, E.A. (2009). *Fruitless, doublesex* and the genetics of social behavior in *Drosophila melanogaster*. Curr. Opin. Neurobiol. *19*, 200–206.
2. Rideout, E.J., Dornan, A.J., Neville, M.C., Eadie, S., and Goodwin, S.F. (2010). Control of sexual differentiation and behavior by the *doublesex* gene in *Drosophila melanogaster*. Nat. Neurosci. *13*, 458–466.
3. Rezaval, C., Pavlou, H.J., Dornan, A.J., Chan, Y.-B., Kravitz, E.A., and Goodwin, S.F. (2012). Neuronal circuitry underlying *Drosophila* female postmating behavioral responses. Curr. Biol. *22*, 1155–1165.
4. Avila, F.W., Sirot, L.K., LaFlamme, B.A., Rubinstein, C.D., and Wolfner, M.F. (2011). Insect seminal fluid proteins: identification and function. Annu. Rev. Entomol. *56*, 21–40.
5. Chen, P.S., Stumm-Zollinger, E., Aigaki, T., Balmer, J., Bienz, M., and Boehlen, P. (1988). A male accessory gland peptide that regulates reproductive behavior of females D. melanogaster. Cell *54*, 291–298.
6. Liu, H., and Kubli, E. (2003). Sex-peptide is the molecular basis of the sperm effect in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA *100*, 9929–9933.
7. Kubli, E. (2003). Sex-peptides, seminal peptides of the Drosophila male. Cell Mol. Life Sci. *60*, 1669–1704.
8. Kubli, E., and Bopp, D. (2010). Sex bei den Insekten. Von Jungfrauen, Liebesgesängen, chemischen Keuschheitsgürteln und Hermaphroditen. Hsg. Naturforschende Ges. Zürich, Jg. *155*, 1–82.
9. Cognigni, P., Bailey, A.P., and Miguel-Aliaga, I. (2011). Enteric neurons and systemic signals couple nutritional and reproductive status with intestinal homeostasis. Cell Metab. *13*, 92–104.
10. Peng, J., Chen, S., Büsser, S., Liu, H., Honegger, T., and Kubli, E. (2005). Gradual release of sperm-bound sex-peptide controls female postmating behavior in Drosophila. Curr. Biol. *15*, 207–213.
11. Domanitskaya, E., Liu, H., Chen, S., and Kubli, E. (2007). The hydroxyproline motif of male sex peptide elicits the innate immune response in Drosophila females. FEBS J. *274*, 5659–5668.
12. Moehle, K., Freund, A., Kubli, E., and Robinson, J.A. (2011). NMR studies of the solution conformation of the sex peptide from *Drosophila melanogaster*. FEBS J. *585*, 1197–1202.
13. Yapici, N., Kim, Y.-J., Ribeiro, C., and Dickson, B.J. (2008). A receptor that mediates the post-mating switch in *Drosophila* reproductive behaviour. Nature *451*, 33–37.
14. Häsemeyer, M., Yapici, N., Heberlein, U., and Dickson, B.J. (2009). Sensory neurons in the *Drosophila* genital tract regulate female reproductive behavior. Neuron *61*, 511–518.
15. Yang, C., Rumpf, S., Xiang, Y., Gordon, M., Song, W., Jan, L.Y., and Jan, Y.-N. (2009). Control of the postmating behavioral switch in *Drosophila* females by internal sensory neurons. Neuron *61*, 519–526.
16. Soller, M., Haussmann, I.U., Hollmann, M., Choffat, Y., White, K., Kubli, E., and Schäfer, M.A. (2006). Sex-Peptide-regulated female sexual behavior requires a subset of ascending ventral nerve cord neurons. Curr. Biol. *16*, 1771–1782.
17. Nakayama, S., Kaiser, K., and Aigaki, T. (1997). Ectopic expression of sex-peptide in a variety of tissues in Drosophila females using the P(GAL4)enhancer-trap system. Mol. Gen. Genet. *254*, 449–455.
18. Aigaki, T., Fleischmann, I., Chen, P.S., and Kubli, E. (1991). Ectopic expression of sex-peptide alters reproductive behavior of female *D. melanogaster*. Neuron *7*, 557–563.
19. Pilpel, N., Nezer, I., Applebaum, S., and Heifetz, Y. (2008). Mating increases trypsin in female *Drosophila* hemolymph. Insect Biochem. Mol. Biol. *38*, 320–330.
20. Clyne, J.D., and Miesenböck, G. (2009). Postcoital finesse. Neuron *61*, 491–493.

Institute of Molecular Life Sciences, University of Zurich-Irchel, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.
E-mail: ekubli@zool.uzh.ch, daniel.bopp@imls.uzh.ch

http://dx.doi.org/10.1016/j.cub.2012.04.058

Cancer and Inflammation: An Aspirin a Day Keeps the Cancer at Bay

Live imaging of the interactions between oncogene-transformed cells and leukocytes in zebrafish reveals that PGE_2 promotes the survival and proliferation of cancer cells. Non-steroid anti-inflammatory drugs, like aspirin, are the effective inhibitors of PGE_2 production and could be used with other anti-tumor agents in the treatment of cancer.

Marina Mione[1] and Leonard I. Zon[2]

The relationship between cancer and inflammation has been a hot subject in research and medicine for centuries. The original observations by Galenius (2000 b.C), Virchow [1] and Dvorak [2] all suggested a close link between inflammation and cancer. As leukocytes are the first line of defense in immune responses, their presence in tumors has been hypothesized to have anti-cancer activity. In recent years, it has become clear, however, that the majority of tumor-associated leukocytes are there to promote tumor growth, tumor angiogenesis, invasion and metastasis. Two years ago, in a paper published in *PLoS Biology*, Feng *et al.* [3] reported that oncogene-transformed cells are able to attract leukocytes immediately after transformation, demonstrating an early trophic support provided by leukocytes to growing tumors. In a new study reported in this issue of *Current Biology*, Feng *et al.* [4], using the same cancer model, now identify the signal that leukocytes provide to cancer cells to promote their survival.

To address these questions, Feng *et al.* [4] used genetic tools and chemical inhibitors to image cancer and inflammation in transparent zebrafish larvae. These authors employed a novel model of oncogene-induced transformation that targets a population of very superficial cells (the mucous-producing cells of the skin). These single cells, closely related to sebaceous gland mucous

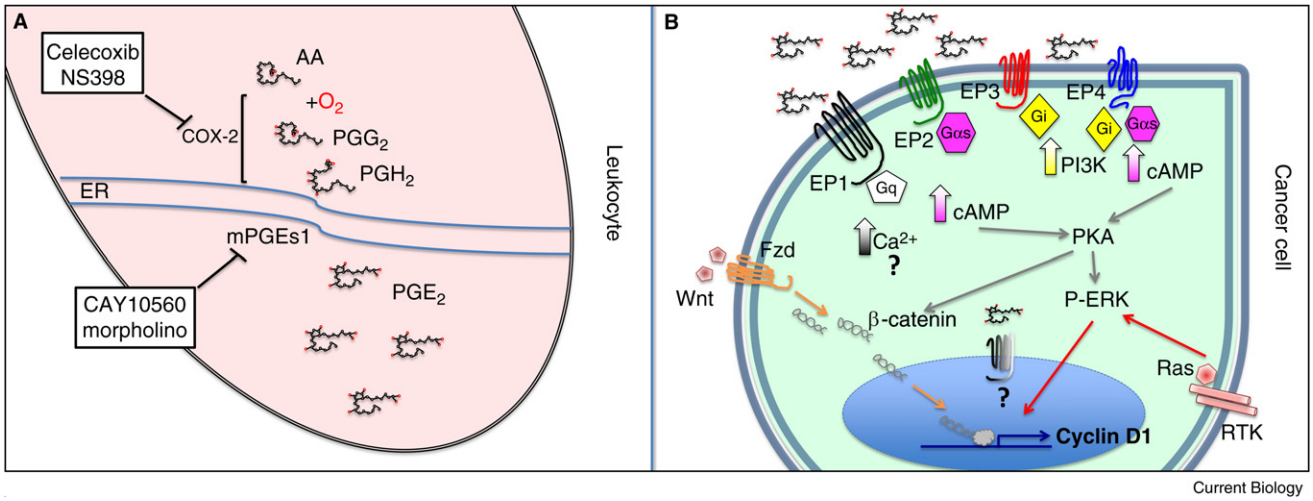

Figure 1. PGE_2 produced by leukocytes near cancer cells acts as a pro-survival signal through the EP1 receptor.

(A) The pathways involved in eicosanoid metabolism in the cytoplasm of a leukocyte approaching a cancer cell are shown, together with the chemical or genetic (morpholino) inhibitors that block specific enzymes. (B) Different PGE_2 receptors (EP1–4) and their association with specific G-protein subunits lead to the activation of different pathways. Protein kinase A (PKA) activation downstream of EP2 or EP4 signaling stabilizes β-catenin (Wnt) and P-ERK (Ras) signals to increase proliferation. EP1 receptors were also found in a perinuclear compartment, where other, as yet unknown mechanisms may lead to transcriptional and epigenetic changes. See text for details and references.

cells in mammals and sparse in the epidermis of zebrafish larvae, respond to oncogene expression by rapid, uncontrolled proliferation [5]. In their previous study, Feng *et al.* [3] had documented that the ability of transformed cells to attract leukocytes through a H_2O_2 signal was beneficial to tumor cells, given that, in the absence of leukocytes, transformed cells did not proliferate and instead underwent apoptosis. The big question that remained unsolved in this earlier study was the nature of the trophic signal released by leukocytes that permitted growth of the transformed cells. In the new work, the search for the trophic signal ended with an interesting candidate, the eicosanoid prostaglandin E_2 (PGE_2), which has been involved in inflammation and cancer (reviewed in [6]) and has a long history of helping cancer cells to survive, proliferate and invade [7]. Besides confirming the 'usual suspect' in a novel model of cancer-induced inflammation, the strongest points of the study lie in the use of combined genetic approaches and small chemical inhibitors to confirm the involvement of PGE_2 and its receptor E-prostanoid 1 (EP1) in the trophic support to transformed cells.

The authors use chemical inhibitors and morpholinos to knock down the enzymatic activities producing PGE_2 and pinpoint the specific pathway — namely microsomal rather than cytoplasmic PGEs — used by leukocytes to produce PGE_2 in the vicinity of Ras-transformed cells (Figure 1A). To overcome the problem of early lethality due to morpholino-mediated ablation of microsomal PGEs, they 'rescued' PGE_2 signaling during the first 9 hours of development by incubating embryos with the long-acting derivative of PGE_2, 16,16-dimethyl-PGE_2 ($dmPGE_2$). This trick allowed them to study the effects of removing PGE_2 when oncogene-transformed cells most needed it, i.e. from the very beginning of transformation. Elegant lineage ablation studies and live observations in neutrophil- and macrophage-specific transgenic lines shed light on which of the two leukocyte cell populations contribute trophic PGE_2 to cancer cells. The answer is that PGE_2 is the only trophic signal produced by macrophages, whereas neutrophils contribute additional as yet unknown trophic products. Because of these detailed characterizations of the enzymatic pathway and of the innate immune cell populations involved, the study is likely to provide significant hints for advancing the search for specific drugs targeting the inflammation–cancer link.

Several questions are raised by this study and remain unresolved, with the exciting possibility of using the same or similar models to find the answers. First of all, how is PGE_2, signaling through the EP1 receptor, sustaining cancer growth? There are studies reporting a role of PGE_2 in promoting Akt phosphorylation in neuroblastoma cells through calcium waves and cyclic AMP (cAMP) production [8] through different PGE_2 receptors. The four EP receptors (EP1–4) have multiple localizations (being found in the cell membrane, cytoplasm or nucleus), each related to specific downstream pathways in PGE_2 signaling (Figure 1B). Although these receptors are not mutually exclusive, their combinatorial expression or activation may lead to different outcomes. The possibility of using an *in vivo* model equipped with a wealth of genetic and chemical biology tools to tease apart the roles of different EP receptors in cancer is extremely exciting.

A recent report attributed to PGE_2 the repression of the DNA demethylases Dnmt1 and Dnmt3b and the corresponding increase in methylation of tumor suppressors in $Apc^{Min/+}$ mice [9] as a mechanism to promote cancer. These mice are well known to PGE_2 aficionados as it was previously shown that lifetime administration of aspirin to these mice suppressed their intestinal and mammary tumors [10]. However, the mechanisms of Dnmt1/Dnmt3b

repression by PGE_2 have not been clarified.

The $Apc^{Min/+}$ mice provide a model for familial polyposis, a fairly common disease associated with APC mutations and cancer predisposition. Here the multiple adenomas forming in the colon of affected patients are sustained by increased Wnt signaling. Non-steroid anti-inflammatory drugs (NSAIDs) are commonly used for the treatment of familial polyposis, and reduction of PGE_2 levels with cyclo-oxygenase (COX) inhibitors has proven effective in suppressing increased Wnt signaling [11].

One of the most tantalizing reports on the cancer-promoting function of PGE_2 is that of the activation of a Ras–MAPK–P-ERK cascade in APC^{Min} mice bearing intestinal adenomas [12]. This is due to a self-amplifying loop that mimics activated Ras and relies on the induction of COX-2 in adenoma cells. It is possible that PGE_2, locally produced by leukocytes, may have the same effect as the externally provided PGE_2 on tumor cells. Clearly, dissecting the different mechanisms through which PGE_2 ensures support to cancer cells may open up new avenues for intervention.

Another question is related to the expression of the PGE_2 receptors. It would be useful to know whether EP1 and other receptors are induced by the oncogene, as a means to provide transformed cells with the ability to respond to leukocyte PGE_2. The implications of Ras-induced EP1 receptor expression are multiple. Besides representing an early marker of transformation, its selective blockage may have amplified responses in cancer prevention as this may be the key point in the PGE_2–Ras activation loop. This loop is mostly mediated by a cAMP response induced by EP2/EP4 activation (Figure 1B), whereas early transformed cells in this model seem to express nuclear EP1. How do PGE_2 nuclear receptors regulate transcription or other nuclear events? One possibility is the convergence of EP1-mediated signals on Wnt target genes, which are induced by nuclear β-catenin accumulation. Indeed, the same EP2/EP4-mediated PKA and cAMP accumulation, which sustains the PGE_2–Ras loop, is responsible for further stabilization of β-catenin [11] downstream of PGE_2 signaling (Figure 1B).

Can we prevent cancer by targeting the pathways downstream of PGE_2? While the overlapping molecular pathways described earlier provide experimental support for this possibility, the 'proof of principle' that targeting innate inflammatory responses in cancer will have anti-tumor effects comes from clinical evidence. Treating chronic inflammation in patients often led to regression of associated cancer, and long-term supply of regular aspirin or other NSAID therapies for the prevention of cardiovascular disorders strongly decreased the incidence of cancer in a large cohort study [13]. In a mouse model of acute myeloid leukemia, the reversible COX inhibitor indomethacin was shown to strongly reduce leukemia-initiating cells through repression of Wnt–β-catenin signaling [14]. NSAIDs function by inhibiting COX-1 and COX-2, which are responsible for the production of all prostaglandins from fatty acids (Figure 1A), but the processes that are affected by a reduction of prostaglandin levels are too numerous to be specific for the cancer–inflammation axis. For example, prostaglandins regulate hormonal activities, coagulation, angiogenesis, vasodilation [15] and hematopoietic stem cell homing and transplantation [16], just to mention a few.

The wide range of actions of NSAIDs explains the decision not to pursue a clinical trial in cancer patients, in spite of the impressive clinical data gathered by Rothwell *et al.* [13], which revealed a reduction by 30–60% of the 20-year risk of cancer death. Most importantly, the reasons for the premature drop in the use of NSAIDs in cancer prevention came from the dramatic outcome of gastrointestinal bleeding and increased thrombocytic events that accompanied the long-term usage of NSAIDs and specific COX-2 inhibitor derivatives in cardiovascular disease prevention trials [17]. Studies like the report by Feng *et al.* [4] will contribute a great deal to develop cancer-specific anti-PGE_2 therapies.

Ultimately, this latest report from Feng *et al.* [4] provides a novel perspective on inflammation and cancer: usually this complex relationship has been tackled from the point of view of infectious agents as cancer inducers (viruses or bacteria through inflammation) or cancer co-factors (inflammation). Here, cancer-initiating cells (expressing an oncogene) are inflammatory (most likely in advanced cancer [18]), even before any morphological or behavioral difference from their untransformed siblings becomes apparent. The new study also provides the hope that altering prostaglandin levels can help prevent cancer.

References

1. Virchow, R. (1863). Aetiologie der neoplastischen Geschwulste/Pathogenie der neoplastischen Geschwulste (Berlin, Germany: Verlag von August Hirschwald).
2. Dvorak, H.F. (1986). Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N. Engl. J. Med. *315*, 1650–1659.
3. Feng, Y., Santoriello, C., Mione, M., Hurlstone, A., and Martin, P. (2010). Live imaging of innate immune cell sensing of transformed cells in zebrafish larvae: parallels between tumor initiation and wound inflammation. PLoS Biol. *8*, e1000562.
4. Feng, Y., Renshaw, S., and Martin, P. (2012). Live imaging of tumour initiation in zebrafish larvae reveals a trophic role for leukocyte-derived PGE2. Curr. Biol. *22*, 1253–1259.
5. Santoriello, C., Gennaro, E., Anelli, V., Distel, M., Kelly, A., Koster, R.W., Hurlstone, A., and Mione, M. (2010). Kita driven expression of oncogenic HRAS leads to early onset and highly penetrant melanoma in zebrafish. PLoS One *5*, e15170.
6. Trinchieri, G. (2011). Innate inflammation and cancer: Is it time for cancer prevention? F1000. Med. Rep. *3*, 11.
7. Menter, D.G., and Dubois, R.N. (2012). Prostaglandins in cancer cell adhesion, migration, and invasion. Int. J. Cell Biol. *2012*, 723419.
8. Rasmuson, A., Kock, A., Fuskevag, O.M., Kruspig, B., Simon-Santamaria, J., Gogvadze, V., Johnsen, J.I., Kogner, P., and Sveinbjornsson, B. (2012). Autocrine prostaglandin E2 signaling promotes tumor cell survival and proliferation in childhood neuroblastoma. PLoS One *7*, e29331.
9. Xia, D., Wang, D., Kim, S.H., Katoh, H., and DuBois, R.N. (2012). Prostaglandin E2 promotes intestinal tumor growth via DNA methylation. Nat. Med. *18*, 224–226.
10. Sansom, O.J., Stark, L.A., Dunlop, M.G., and Clarke, A.R. (2001). Suppression of intestinal and mammary neoplasia by lifetime administration of aspirin in Apc(Min/+) and Apc(Min/+), Msh2(−/−) mice. Cancer Res. *61*, 7060–7064.
11. Goessling, W., North, T.E., Loewer, S., Lord, A.M., Lee, S., Stoick-Cooper, C.L., Weidinger, G., Puder, M., Daley, G.Q., Moon, R.T., *et al.* (2009). Genetic interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration. Cell *136*, 1136–1147.
12. Wang, D., Buchanan, F.G., Wang, H., Dey, S.K., and DuBois, R.N. (2005). Prostaglandin E2 enhances intestinal adenoma growth via activation of the Ras-mitogen-activated protein kinase cascade. Cancer Res. *65*, 1822–1829.
13. Rothwell, P.M., Fowkes, F.G., Belch, J.F., Ogawa, H., Warlow, C.P., and Meade, T.W. (2011). Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet *377*, 31–41.
14. Wang, Y., Krivtsov, A.V., Sinha, A.U., North, T.E., Goessling, W., Feng, Z., Zon, L.I., and Armstrong, S.A. (2010). The Wnt/beta-catenin pathway is required for the development of leukemia stem cells in AML. Science *327*, 1650–1653.

15. Wang, D., and Dubois, R.N. (2010). Eicosanoids and cancer. Nat. Rev. Cancer *10*, 181–193.
16. North, T.E., Goessling, W., Walkley, C.R., Lengerke, C., Kopani, K.R., Lord, A.M., Weber, G.J., Bowman, T.V., Jang, I.H., Grosser, T., *et al*. (2007). Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. Nature *447*, 1007–1011.
17. Wolfe, M.M., Lichtenstein, D.R., and Singh, G. (1999). Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N. Engl. J. Med. *340*, 1888–1899.
18. Colotta, F., Allavena, P., Sica, A., Garlanda, C., and Mantovani, A. (2009). Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis *30*, 1073–1081.

[1]Institute of Toxicology and Genetics, Karlsruhe Institute of Technology, Karlsruhe, Germany. [2]Stem Cell Program and Division of Hematology/Oncology, Children's Hospital and Dana Farber Cancer Institute, Howard Hughes Medical Institute, Harvard Stem Cell Institute, Harvard Medical School, Boston, MA, USA.
E-mail: Maria.Mione@kit.edu, zon@enders.tch.harvard.edu

http://dx.doi.org/10.1016/j.cub.2012.05.037

Evolution: Sociality as a Driver of Unorthodox Reproduction

An unusual reproductive system was discovered in desert ants, in which daughter queens are produced asexually via parthenogenesis, whereas workers develop from hybrid crosses between genetically divergent lineages. The system appears to be doomed to extinction.

Tanja Schwander[1,*] and Laurent Keller[2]

Although species are commonly defined as groups of shared reproduction, many species do form hybrids in nature. In most cases, these hybrids are infertile and perish in one generation, but some hybrid species have escaped this constraint by acquiring unusual reproductive modes. Several hybrid species of fish, stick insects and frogs consist only of females and rely on males of closely related species to fertilize their eggs. When such hybrid females produce eggs themselves, they selectively discard the chromosomes inherited from their father to only transmit the genetic material of their mother [1]. In such cases, referred to as 'hybridogenesis', females thus asexually transmit their maternal genome to the next generation, while males are only used as 'sperm donors' for the production of the soma.

A new paper in this issue of *Current Biology* [2] now describes a social version of hybridogenesis. In the desert ant *Cataglyphis hispanica* (Figure 1A), queens use alternative modes of reproduction to produce reproductive daughter queens and the non-reproductive workers, which are necessary for the maintenance and survival of the colony. All daughter queens are produced asexually via parthenogenesis while workers are sexually produced from hybrid crosses between two genetically distinct lineages. Each of the two lineages has a set of private microsatellite alleles, indicating that they are independently evolving entities with little or no gene flow between them.

Social and Non-Social Hybridogenesis

There are striking similarities between this system of 'social hybridogenesis' and the known systems of (non-social) hybridogenesis. In both systems, sperm is used only to produce individuals (the workers) or cells (somatic cells) that do not contribute to the transmission of genetic material between generations. However, two important features distinguish the system of social hybridogenesis in *C. hispanica* from its non-social version: first, instead of having one species depending on the presence of males of another species to reproduce (normal hybridogenesis), both lineages of *C. hispanica* need each other to obtain the sperm required for the production of workers; second, both *C. hispanica* lineages still produce males while males are entirely absent from non-social hybridogenetic species.

Although the *C. hispanica* reproductive system most closely mirrors non-social hybridogenesis, the term social hybridogenesis was originally coined for a reproductive system in *Pogonomyrmex* harvester ants [3,4] where at least eight divergent lineages co-occur in specific lineage-pairs [5]. Queens mate multiply with males of their own and of the alternative lineage; offspring produced from same-lineage matings always develop into queens, whereas inter-lineage hybrids develop into workers [3,4,6,7]. Thus, while in both *C. hispanica* and *Pogonomyrmex* workers are produced from crosses between divergent lineages, the two systems differ in how queens are produced: by normal sexual reproduction in *Pogonomyrmex* and via parthenogenesis in *C. hispanica*.

Four additional ant species have been shown to have reproductive systems relying on hybridization between species or lineages for worker production. Similarly to *Pogonomyrmex*, *Solenopsis xyloni* workers are produced from matings with a different species (*S. geminata*) while within-species fertilizations give rise to new queens [8]. The difference is that *S. xylony* colonies comprise multiple queens each mated with a single male. Thus, queens produce exclusively new queens or workers depending on the male they mated with. In the three remaining species — *Wasmannia auropunctata*, *Vollenhovia emeryi* and *Paratrechina longicornis* — the situation is even more baroque [9–11]. New queens are also produced via parthenogenesis and workers are produced sexually via hybrid crosses. However, the hybrid crosses take place between the maternal lineages and a species consisting exclusively of males. These males reproduce by using females as 'egg donors': instead of developing from unfertilized eggs, as is usually the case in ants, the sperm most likely eliminates the maternal